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Possible Involvement of Exon 31 Alternative Splicing in Phenotype and Severity of Epidermolysis Bullosa Caused by Mutations in *PLEC1*

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TO THE EDITOR

Epidermolysis bullosa constitutes a group of phenotypically diverse genodermatoses which manifest with blistering and erosions of the skin and mucous membranes (Fine *et al.*, 2000). Recent advances in epidermolysis bullosa research have allowed the identification of mutations in 10 different genes, which account for the clinical heterogeneity in epidermolysis bullosa (Pulkkinen and Uitto, 1999).

Mutations in the plectin gene (*PLEC1*) are generally thought to cause

Abbreviations: EBS, epidermolysis bullosa simplex; EBS-MD, epidermolysis bullosa simplex associated with muscular dystrophy; EBS-PA, epidermolysis bullosa simplex associated with pyloric atresia; PTC, premature termination codon

epidermolysis bullosa simplex (EBS) associated with muscular dystrophy. The majority of these cases are characterized by generalized blistering and muscular dystrophy (McLean *et al.*, 1996; Smith *et al.*, 1996; Shimizu *et al.*, 1999). Muscle weakness is first observed during the latter part of the first decade of life. However, we and other groups have recently demonstrated that lethal EBS cases with pyloric atresia (EBS-PA) also result from mutations in *PLEC1* (Nakamura *et al.*, 2005; Pfendner and Uitto, 2005b). Seven cases of this new variant of EBS have been reported so far (Pfendner *et al.*, 2005a). These patients manifest with cutaneous blisters, aplasia cutis congenital (severe localized absence of skin), and pyloric atresia, which rapidly result in the patient's demise, often soon after birth. This study reports two cases with defective plectin expression that show EBS-PA and EBS with muscular dystrophy (EBS-MD). Furthermore, based on data mining from the *PLEC1* mutation database, we suggest the possible involvement of exon 31 in alternative splicing that may alleviate the phenotypic severity of epidermolysis bullosa cases caused by mutations in the plectin gene.

Case 1 was a 4-month-old boy with skin fragility from birth. There was no other family history of skin fragility (Figure 1a). Generalized blisters and erosions were found over his entire body. He was diagnosed as suffering from pyloric atresia by routine abdominal X-ray. Case 2 was a 49-year-old female with skin fragility from birth (Figure 1b). Family tree showed a history of consanguinity, although there was no other family history of skin fragility. She is now bedridden and requires a respirator owing to progressive muscular dystrophy. Some blisters and erosions were observed on her trunk and extremities.

An immunohistochemical study using mAbs to a range of basement membrane zone component proteins was performed. Immunoreactivity against plectin rod domain was markedly attenuated in cases 1 and 2 (Figure 1c). Immunostaining for other basement membrane zone proteins including bullous pemphigoid antigens 1 and 2, the α6 and β4 integrins, laminin 5, and type VII collagens were normal (data not shown). Direct nucleotide sequencing of *PLEC1* demonstrated that case 1 harbored novel heterogeneous premature termination codon (PTC) mutations Q2466X in exon 31 and Q2545X in exon 32, whereas case 2 harbored a novel homozygous PTC mutation, Q1450X, in exon 31 (Figure 2a). Informed consent was obtained from all individual subjects in this study. The protocols were approved by the Ethical Committee at Hokkaido University Graduate School of Medicine. This study was conducted according to the Declaration of Helsinki Principles.

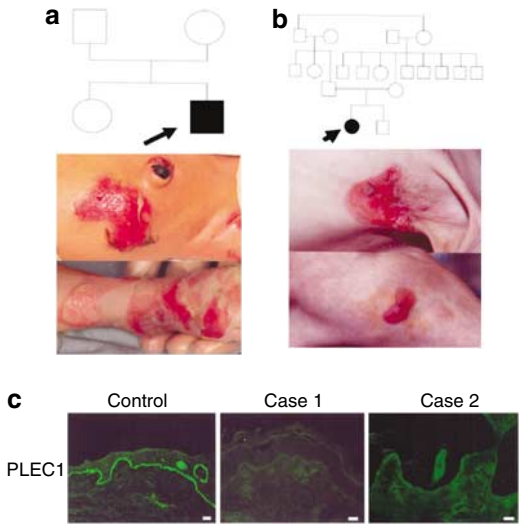

Figure 1. Family trees, clinical findings and plectin expression. (**a**) Case 1 was a 4-month-old boy who has exhibited skin fragility since birth. There was no family history of skin fragility. Blisters and erosions were scattered over his whole body and oral mucous membranes were also affected. Healing occurred without scarring and milia formation. He was diagnosed as suffering from pyloric atresia by routine abdominal X-ray. On the fourth day after birth, pyloroplasty was performed. He suffered from aspiration pneumonia and impairment of weight gain before the age of 6 months, but afterwards the volume of milk taken increased and blister formation steadily lessened. No muscular and neurological findings were observed. (**b**) Case 2 was a 49-year-old female with skin fragility from birth. Several blisters and erosions were found on her trunk and extremities. Slight scar formation was seen in some areas. Hypoplasty of her permanent dentition was seen and some nail thickening was observed. Her family tree showed a history of consanguinity. Although mild blister formation continued, muscle symptoms had never been found, until muscle weakness on the arms was first noted at the age of 19 years. Muscle weakness gradually progressed, but she was able to perform routine activities. However, she could not walk at the age of 38 years, owing to widespread muscular atrophy. She is now confined to a bed and breathing is assisted by a respirator. (**c**) Direct immunofluorescence analysis using mAb HD1-121 against plectin (a kind gift from Dr Owaribe K, Nogoya University) demonstrated that immunoreactivities were markedly attenuated in cases 1 and 2 compared with normal control. Bar = 50 μm.

Cases 1 and 2 demonstrated extracutaneous involvement including PA and MD, respectively. We believe that the clinical features and course of case 2 were typical of EBS-MD. Although all seven previous cases of EBS-PA showed a severe, lethal clinical course (Pfendner *et al.*, 2005a), case 1 was much milder than those cases and even showed some clinical improvement over time, so this is the first case of EBS-PA with a relatively moderate phenotype.

The precise pathomechanism causing the clinical differences between EBS-MD and EBS-PA has not yet been elucidated (Nakamura *et al.*, 2005; Pfendner *et al.*, 2005a; Pfendner and Uitto, 2005b). The *PLEC1* mutation database has accumulated almost 40 *PLEC1* mutations from 22 cases of EBS-MD and seven cases of EBS-PA (Pfendner *et al.*, 2005a, McMillan *et al.*, 2007). We have carefully re-examined genotype–phenotype correlations in EBS-MD and EBS-PA. The plectin database contains many homozygous mutations and we plotted only homozygous PTC mutations in order to minimize the effect of the expression difference

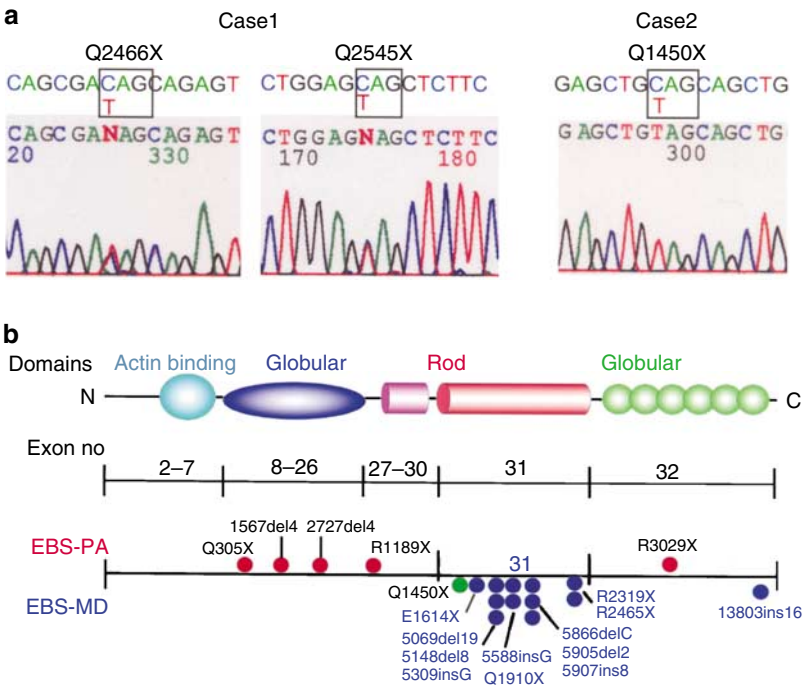

Figure 2. *PLEC1* mutations and significance of exon 31. (**a**) Genomic DNA was obtained from both cases and the parents. The mutation detection strategy was performed after PCR amplification of all exons and intron-exon borders, followed by direct automated nucleotide sequencing. The genomic DNA nucleotides, the complementary DNA nucleotides and the amino acids of the protein were numbered based on the previous sequence information (GenBank accession no. AH003623) (McLean *et al.*, 1996). Case 1 demonstrated heterozygous nonsense mutations. The maternal nonsense mutation was a C→T transition at nucleotide c.7396 of complementary DNA in exon 31, resulting in the substitution of a glutamine (CAG) at position 2466 with a stop codon (TAG) (Q2466X). The other paternal nonsense mutation was also a C→T transition at nucleotide c.7633 of complementary DNA in exon 32, resulting in the substitution of a glutamine (CAG) at position 2545 with a stop codon (TAG) (Q2545X). Case 2 showed homozygous nonsense mutations, which was a C→T transition at nucleotide c.4348 of complementary DNA in exon 31, resulting in the substitution of a glutamine (CAG) at position 1450 with a stop codon (TAG) (Q1450X). (**b**) The plectin database shows many homozygous mutations and we plotted the position of only homozygous PTC mutations. Interestingly, homozygous PTC mutations associated with EBS-MD (blue circles) are located in exon 31 except for one mattation13803ins16 whereas those with EBS-PA (red circles) are in other parts of the gene except exon 31. Q1450X (green circle) is the present case. Amino-terminal actin binding domain, amino-terminal globular domain, rod domain and carboxyl-terminal globular domain are shown. Positions of exons are indicated by numbers (exon no).

between the two *PLEC1* alleles. Interestingly, homozygous PTC mutations associated with EBS and MD are located in exon 31 except for one (13803ins16), whereas those with EBS with PA are located in parts of the gene other than exon 31 (Figure 2b). Thus, the plectin database suggested that EBS-MD and EBS-PA were associated with mutations in exon 31 and other than in exon 31, respectively.

Analysis of the murine plectin gene showed that alternative splicing resulted in more than 16 plectin variants and that tissue-specific expression of these variants was different (Fuchs *et al.*, 1999; Rezniczek *et al.*, 2003). This leads to the possibility that PLEC1 alternative splicing affects the severity of blistering and extracutaneous manifestation. Plectin comprises a central rod domain with a α-helical coiled-coil structure and large flanking amino-and carboxyl-terminal globular domains (Liu *et al.*, 1996; Wiche, 1998). Recently, one alternate splice messenger RNA transcript which lacks exon 31 encoding the central rod domain was identified in multiple rat tissues (Elliott *et al.*, 1997; Steinboeck and Kristufek (2005)). In fact, skin fragility in patients with *PLEC1* mutations was less severe than that observed in plectin-deficient mice. As most of *PLEC1* mutations are located within the rod domain that was not present in the smaller splice variant, this variant might in part compensate for the loss of the canonical (full length) plectin expression in humans (Litjens *et al.*, 2006). To understand the expression of the rodless alternative spliced form in various human tissues and cells, we performed plectin domain-specific RT-PCR, which indicated that human cells also express the rodless isoform at various levels (data not shown).

A combination of our mutations and plectin expression results with the above mutation database, suggests that exon 31 alternate splicing may restore the *PLEC1* open-reading frame in EBS-MD patients with PTC mutations in exon 31, and partially rescue the phenotype. Therefore, we suggest that EBS-MD caused by mutations in exon 31 demonstrates a milder phenotype than EBS-PA caused by non-exon 31 *PLEC1* mutations. Furthermore, the relatively moderate phenotype of case 1 with non-lethal EBS-PA might result from one mutation associated with exon 31.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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A Novel *GJB2* Mutation p.Asn54His in a Patient with Palmoplantar Keratoderma, Sensorineural Hearing Loss and Knuckle Pads

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TO THE EDITOR

Mutations in the *GJB2* gene encoding connexin26 are the major cause of autosomal-recessive or -dominant non-syndromic congenital sensorineural hearing loss (SNHL) (Kelsell *et al.*, 1997; Kenneson *et al.*, 2002; refer to the connexin-deafness homepage at http://davinci.crg.es/deafness/). In addition, connexin26 mutations have been identified in autosomal-dominant syndromic congenital SNHL with palmoplantar keratoderma (PPK) (Maestrini *et al.*, 1999; Richard *et al.*, 2002, 2004; Brown *et al.*, 2003; van Steensel *et al.*, 2004; Arita *et al.*, 2006). We have encountered a Japanese boy with PPK, knuckle pads and congenital SNHL and *GJB2* mutation analysis revealed a novel mutation p.Asn54His.

The patient was a 12-year-old Japanese boy with PPK, knuckle pads on the fingers and severe SNHL. He had a congenital onset of profound bilateral SNHL. At 1 year of age, he developed PPK and knuckle pads. There was no familial history of skin disorders or auditory dysfunction. At age 12, moderate PPK was seen. Knuckle pads were apparent on all his fingers (Figure 1a and b). Acneiform follicular keratotic papules were seen on his forehead and face, although these acneiform papules might just be acne. No mutilation (pseudoainhum) was seen on the fingers. Nails, hair, and teeth were normal and no leukonychia was observed. Ophthalmologic examination revealed no apparent abnormality.

Abbreviations: PPK, palmoplantar keratoderma; SNHL, sensorineural hearing loss

The medical ethical committee at Hokkaido University approved all studies described below. The study was conducted according to the Declaration of Helsinki Principles. Participants or their legal guardian gave their written informed consent. The coding region of *GJB2* (Genbank accession no. NM 004004) was amplified from genomic DNA by PCR, as described previously (Richard *et al.*, 1998). Direct sequencing of the patient's PCR products revealed that the patient was a heterozygote for a novel missense mutation p.Asn54His (A to C substitution at nucleotide position 160: asparagine 54 (AAC) to histidine (CAC)) in *GJB2* (Figure 1d), which was not found in his mother. We were unable to obtain a DNA sample from his father. This mutation was not found in 100 normal unrelated Japanese alleles